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TITLE: Molecular Markers of Estrogen Metabolism and Progression from High-Grade Prostatic Intraepithelial Neoplasia (HGPIN) to Prostate Cancer

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14. ABSTRACT: The purpose of this case-control study is to investigate the association between genetic and endocrine markers of estrogen metabolism and prostate cancer progression. Androgens (e.g., testosterone) may be critical in prostate cancer progression, but there is accumulating evidence that estrogens facilitate progress during the later stages of prostate cancer formation 1-4. To explore the role of estrogens in human prostate carcinogenesis, we proposed to investigate the association between genetic and endocrine markers of estrogen metabolism and the detection of high-grade prostatic intraepithelial neoplasia (HGPIN) and stage I/II/III prostate cancer. The first project year included protocol development and IRB approval, and the second year focused on subject recruitment and data collection. The third year focused on recruitment, data collection, and analysis. Specific accomplishments include recruitment of 717 subjects to the protocol (95% of eligibles). We have conducted several analyses looking at the association between genetic variants or obesity and HGPIN or prostate cancer. We have exceeded recruitment goals, and extended interpretation to current public health priority. Further details provided below are in parallel with the statement of work.					
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Table of Contents

Cover.....	1
SF 298.....	2
Table of Contents.....	3
Introduction.....	4
Body.....	5
Key Research Accomplishments.....	18
Reportable Outcomes.....	19
Conclusions.....	20
References.....	21
Appendices.....	N/A

DAMD17-02-1-0139

MOLECULAR MARKERS OF ESTROGEN METABOLISM AND PROGRESSION FROM HIGH-GRADE PROSTATIC INTRAEPITHELIAL NEOPLASIA (HGPIN) TO PROSTATE CANCER

Fourth (Final) Annual Report due February 14, 2006

This study has completed the third and final full year of funded activity. Data collection protocols have been finalized and protocols approved by appropriate IRBs. The majority of work revolved around subject recruitment, data collection, biospecimen collection. Also, significant effort was placed on data management and statistical analyses necessary to achieve specified aims. Accomplishments are described in the following narrative, in parallel with the original Statement of Work.

INTRODUCTION

This work is based on a growing body of laboratory evidence that estrogens contribute to the advancement and detection of prostate cancer. Prostate tumor cells express estrogen receptors, and estrogen exposure may sustain tumor growth as androgen levels decrease with advancing age¹. It has further been shown that not all estrogens behave alike. Relative to 2-hydroxyestrone (2HE), 16HE appears bind with higher affinity to the estrogen receptor⁵, and the relative balance of estrogen metabolites is determined by several enzyme activities. The CYP3A4 and CYP1B1 P-450s are responsible for converting estradiol to the 16HE or 4 HE metabolites, respectively, at the expense of 2HE production. Both CYP1B1 and CYP3A4 are expressed in prostate tissue, a polymorphism of the *CYP3A4* gene in the 5' transcriptional regulatory element (*CYP3A4-V*) has been significantly associated with later age and advanced stage at CaP detection compared to men with the wild-type gene⁶. Similarly, prostate cancer cases may be 3-fold more likely than controls to be homozygous for the *CYP1B1*^{Val} allele⁷. The goal of this New Investigator Award is to conduct a pilot case-control study investigating the association between molecular markers of estrogen metabolism and diagnosis of prostate cancer (stage II/III) and high-grade prostatic intraepithelial neoplasia (HGPIN), the purported precursor pathologic state to prostate cancer. We will recruit 45 men with HGPIN, 45 prostate cancer cases, and 45 healthy controls free of prostate cancer at biopsy. Genetic polymorphisms in CYP3A4 and CYP1B1 associated with estrogen metabolism will be determined from blood collected from each participant. Data regarding family history of prostate cancer and other risk factors is collected by questionnaire, and habitual dietary intake is measured by in-person interview. We hypothesize that CaP cases will have lower urinary 2HE/16HE and 2HE levels, and higher urinary 16HE, 4HE, E2 and blood E2 levels compared to HGPIN cases or healthy controls. Also, new research finds that prostate cancer is more strongly associated with obesity than previously thought⁸⁻¹⁰. In men, body fat converts androgens to estrogens, and therefore an investigation of body fat and obesity on prostate cancer has direct relevance to the aims of this study.

With completion of the fourth year of this award, we have met and built upon the 1-Year, 2-Year,

and 3-Year objectives specified in our statement of work, including the obtainment of IRB approval, development of questionnaires and other data collection tools. We have expanded our recruitment base to include the Veteran's Hospitals in Nashville and Murfreesboro, TN. Recruitment of study participants has required a great deal of work, but we have exceeded initial recruitment estimates. Data from almost 700 subjects has been entered into a computer database, quality checked, and are ready for analyses. DNA from blood has been extracted, 8 genetic polymorphisms evaluated. Original protocols measuring estrogen metabolite have since shown to be unfeasible, however we have made special arrangement with Genova Diagnostics to conduct estrogen metabolite analyses. Based on recent research developments described above, we have added analyses of prostate cancer in the context of obesity.

BODY

Work accomplished or initiated during each year of this NIA encompasses Statement of Work tasks outlined in Stage 1 (Run-In: Months 1-8)) and Stage 2 (Recruitment and Data Collection: Months 9-26)), Stage 3 (Months 12-30), Stage 4 (24-30 months), and Stage 5 (31-36 months).

Stage 1: Run-In Phase: Months 1-8:

1. Develop all questionnaires, pathology record abstraction forms, and data collection protocols

Year 1: All forms were developed specifically for this project. Our risk factor questionnaire measures include: demographics; age; sex; race; marital status; education; number of children; medical history and treatment; family health history of prostate cancer; history of other cancers, exercise frequency; and smoking status. (Appendix). Our dietary assessment instrument is the Diet History Questionnaire, recently developed by the NIH and shown to have favorable reliability and validity to other established dietary assessment instruments. We had a computer-assisted program developed to guide the implementation of our dietary assessment instrument. Our Pathology Record Abstraction form records the clinic sites, dates and clinical results of all PSA, other lab assays, and digital rectal exam results, ultrasound results, biopsy history and results, pathological diagnosis (HGPIN, cancer), and zone and distribution of the disease. Similarly, we record any cancer diagnosis, as well as stage and Gleason scale of cancer. We have developed Body Measurement protocols (Anthropometrics), including the methods of measuring and recording measurement of participant weight, height, sitting height, waist, and hips. Our Biospecimen Collection form records blood collection status for purposes of DNA extraction or serology, and urine specimens for estrogen metabolite measurement. All samples are aliquoted and stored frozen as necessary to meet our research objectives. Medication use is recorded on the Medication Use form. Subjects are instructed to bring all current medications and supplements to the data collection meeting, and name of the drug, dosage, and use patterns are recorded. The Interview Checklist ensures that all assigned tasks required during the interview are completed, including a review of eligibility, completion of all data collection protocols, and proper consent of participant.

These documents have been submitted to the DOD during prior annual reports.

2. Hire a Research Nurse

Year 1: For this project, Saundra Motley, RN, was hired to serve as Project Manager. Ms. Motley is responsible for the day-to-day administration. She also has been trained to collect body size measurements using standard and systematic protocols, as well as in urine and blood collection, sample preparation, and storage protocols.

Year 2: Ms. Motley continued to serve as Project Manager. She is responsible for recruitment, data collection, and day-to-day administration of the project.

Year 3: Ms. Motley continued to serve as Project Manager. She is responsible for recruitment, data collection, and day-to-day administration of the project.

Year 4: Ms. Motley continued to serve as Project Manager. She is responsible for recruitment, data collection, and day to day administration of the project

3. Pre-test questionnaires and forms

Year 1: All instrument materials have been thoroughly reviewed, and final versions of all assessment instruments have been produced.

4. Refine questionnaire protocols, as necessary

Year 1: The Baseline Questionnaire was edited to provide a better organization to the questions. We have improved our assessment of dietary intake by developing portion size guides (Appendix), and the addition of several foods common to the Tennessee population (asparagus, okra, squash). We are conducting a sub-study to determine if completion of the dietary questionnaire at home and with a wife/partner provides substantially different food intake scores compared to our in-person interview protocol. The laptop computer program we had developed for this project initially had several 'glitches', but is now working without errors or failures.

5. Finalize IRB application and consent form, if necessary

Year 1: IRB approved consent forms have been obtained from Vanderbilt University, the VA (Nashville), and the US AMRMC (Appendix).

Year 2: We have started to expand the recruitment base to the VA in Murfreesboro TN. Also, we are in negotiations with a large private urology practice to start recruitment at this site.

Year 3: We successfully recruited from all clinical sites. This included the clinics at Vanderbilt University, the VA hospitals in Nashville and Murfreesboro, TN, and a large private urology clinic in Nashville.

Year 4: We maintained recruited from all clinical sites, with particular focus on a large private urology clinic in Nashville. This clinic had very high patient attendance levels, and provided a very productive recruitment site to meet our research needs.

6. Develop the study data management systems, including the combination of Teleform, Microsoft Access, Epi-Info, and SAS

Year 1: Our data management system continues to develop, as we recognize and incorporate new technologists into our study. We are able to rapidly identify potentially eligible men receiving care at one of the urology cancer centers through newly implemented scheduling and patient monitoring software in the clinic. This will greatly increase our efficiency to recruit urology patients in the future. New data collection protocols have been developed to fully utilize all resources. Dietary assessment data are entered directly into an ACCESS database using a computer-based dietary assessment interview program created specifically for this project. The ACCESS database may be read by SAS, and merged with other databases for the purposes of analysis. Additional data-entry programs for questionnaires and forms will follow this model, using ACCESS with subsequent transfer into SAS.

Year 2: Data entry programs for all data collection forms have been developed. Data are entered on a periodic basis throughout the year. About 50% of collected data have been key-punched at this time.

Year 3: Data for 489 subjects have been key-punched and quality checked. This includes double-key-punch entry and evaluation of mean and outlier values. Suspicious values have been checked for accuracy.

Year 4: Data for 717 subjects have been key-punched and quality checked. This includes double-key-punch entry and evaluation of mean and outlier values. Suspicious values have been checked for accuracy.

7. Become trained in all clinic-site office and administrative procedures

Year 1: The PI and/or Project Manager have received tours of the urology clinic at Vanderbilt and the VA (Nashville), and understand the operational and administrative procedures of this clinic.

Year 2: The PI and Project Manager have received tours of the urology clinic at the VA (Murfreesboro), and recruitment at this site has started.

8. Hire and Train Project Director in all clinic based procedures

Year 1: See #2 above. The position of Project Director and Research Nurse were efficiently combined under Ms. Motley's position in the study.

Year 2: See #2 above. Ms. Motley continues as Project Coordinator. With expansion of recruitment to the VA at Murfreesboro, TN, she is training a VA research nurse in our data collection protocols, in addition to other study responsibilities. Full recruitment from this VA will begin once training is concluded.

Year 3: See #2 above. Ms. Motley continues as Project Coordinator.

Year 4: See #2 above. Ms. Motley continues as Project Coordinator.

9. Finalize all biological sample collection and storage procedures to be used in the study

Year 1: All biological sample collection and storage procedures for urine and blood cells and serum are finalized. After obtaining consent, we obtain a urine and blood sample, body size measurements, and participants complete the diet questionnaire. Urine is aliquoted to 9 cryovials, and stored at -80°C . One tube of blood, 5ml (EDTA) is collected for DNA extraction. Two 5ml tubes of blood (No anticoagulants) are collected, centrifuged, and the supernate aliquoted for steroid hormone measurement.

Year 2: Biospecimen collection and storage protocols are being implemented according to protocols.

Year 3: Biospecimen collection and storage protocols are being implemented according to protocols.

Year 4: Biospecimen collection and storage protocols are being implemented according to protocols.

10. Establish reliability of all laboratory procedures to be used

Year 1: The genotyping assays for CYP3A4 and CYP1B1 are routine genotyping assays in our group with established reliability. The estrogen metabolite assays have published reliability and validity. Laboratory reliability will be determined at the time the assays are scheduled to run, during Year 2 or Year 3 of the project.

Year 2: A subset of urine samples have been sent to Dr. Fritz Parl for trial analysis of urinary estrogen metabolite levels. Results from this lab trial were not available at the time of this report.

Year 3: Genotyping for CYP1B1 polymorphism is complete. Genotyping for CYP3A4 polymorphism is currently in progress. This genotyping was delayed somewhat due to a misunderstanding in assay protocol between the PI and service lab, but is now in progress and should be complete within 3 weeks.

Year 4: Genotyping for CYP1B1 and CYP3A4 polymorphisms are complete. We have extended the genotyping protocol to include PPAR γ 2 and CYP19 genes, driven by our preliminary results that obesity is a driving force in HGPIN detection. Thus, the genetic analyses have been expanded beyond the original aims of the project.

11. Establish screening procedures for men with HGPIN, prostate cancer patients, or controls.

Year 1: Candidates are identified by collaborators in the urology clinics as potentially eligible for our research study. As part of standard recruitment procedures, we mail an introductory letter and brochure to candidates (Appendix). This is followed by a telephone calls (Appendix for script) intended to answer any questions and evaluate interest in the study. If interested and determined potentially eligible, a meeting is scheduled at the study center. The Project Manager confirms eligibility and obtains informed consent (Appendix for consent form) prior to data collection.

Year 2: Recruitment and eligibility screening procedures were implemented.

Year 3: Recruitment and eligibility screening procedures are maintained.

Year 4: Recruitment and eligibility screening procedures are maintained.

12. Order supplies necessary for biological sample collection

Year 1: All supplies necessary for biological sample collection have been ordered and received.

Year 2: Additional supplies ordered as needed.

Year 3: Additional supplies ordered as needed

Year 4: Additional supplies ordered as needed.

13. Create complete manual of operations

Year 1: We have created a manual of operations to organize and record every procedures and protocol in this study. All items in the Appendix are included in the Manual, as well as a copy of the original grant proposal and correspondence with DOD colleagues.

Year 2: The Manual of Operations continues to be updated as protocols are revised or as administrative documents are received.

Year 3: The Manual of Operations was updated as needed, and now contains a complete record of all protocols, study documents, and procedures

Year 4: The Manual of Operations was updated as needed, and now contains a complete record of all protocols, study documents, and procedures.

Stage 2a: Recruitment of 45 men with high grade intraepithelial neoplasia, 45 men with Stage II/III prostate cancer, and 45 men without CaP or HGPIN at biopsy: Months 8-26

1. Identify 45 men with HGPIN eligible for the study from the urology clinics.

2. Identify 45 men with prostate cancer.

Year 1 and Year 2: Study recruitment started January 22, 2003. At the end of Year 1, we had recruited, consented, and collected complete data from 15 participants: HGPIN (n=3), Prostate Cancer (n=7), Controls (n=5). At the end of Year 2, we have recruited and obtained complete data from 129 subjects: HGPIN (n=12), Prostate Cancer (n=55), Controls (n=62).

Year 3: At this time (Year 3), we have data from 491 patients: HGPIN (n=66), Prostate Cancer (n=180), Controls (n=232), and Suspicious (n=13). Approximately 20 more subjects are in various stages of recruitment and data collection. Thus, we have met our recruitment goal of 45 HGPIN patients, and we will be able to conduct analyses comparing prostate cancer, HGPIN, and Controls on parameters of estrogen exposure. This recruitment success is important, as this now is one of the larger clinical and biospecimen databases of HGPIN patients. We note that

recruitment was initially delayed by approximately 4 months due to IRB requirements, thus extension of recruitment efforts into Year 3 was expected.

Year 4: We have exceeded all recruitment goals, and data and biospecimen repositories now include complete information for 276 cancer cases, 106 HGPIN cases, and 356 controls. Our recruitment rate of eligibles exceeds 95%, a very high success rate in this day and important as we translate our NIA study findings to a full-scale prostate cancer study. This is a pivotal issue, as our success in recruitment has led to several grant applications and the development of manuscripts in progress that address important public health issues.

3. Abstract medical records for health history and pathology data, as described in Methods section of this proposal.

Year 1 and **Year 2**: We have accessed medical records and abstracted relevant prostate cancer pathology information using our pathology data form.

Year 3: We have completed an extensive medical chart review of all subjects. Medical charts were reviewed, data recorded on data abstraction forms, and double-key punched into a computer database. These data include all available pathology information from biopsy. Pathology from prostatectomy patients is included when appropriate.

Year 4: We continue all protocols or medical chart review.

4. Gain informed consent

Years 1-4: All participants are consented in an IRB approved manner

5. Among those who say they are willing to participate, determine eligibility using the criteria described in the Methods Section of this proposal

Years 1-4: Eligibility is determined via telephone interview, and confirmed during the in-person interview.

6. Enroll consecutive eligible men with confirmed HGPIN or prostate cancer.

Year 1-4: Approximately 95% of eligible HGPIN and prostate cancer patients approached for enrollment have enrolled in our study.

7. Ensure that the spot urine samples and blood samples are collected, processed, and stored in a -80°C freezer at the Vanderbilt University

Years 1-4: All urine and blood samples are collected and processed using described protocols, then stored in a -80°C freezer at Vanderbilt University.

8. Collect data on lifestyle, demographics, and health (family and personal history), as outlined in proposal

Years 1-4: Each participant completes a Background Questionnaire, collecting lifestyle, demographic, and medical data. This questionnaire is mailed to participants prior to the

clinical visit, and then reviewed by the Project Manager at that time for completeness. Any blank questions or illogical responses are clarified at that time.

9. Pathology record abstractions will be performed

Years 1-3: The Project Manager abstracts relevant medical and pathology information from the pathology report, and records this information on the Pathology Report Form. This data are periodically key-punched into an ACCESS database.

10. Collect anthropometrics

Years 1-3: We measure weight, height, waist, hips, and sitting height for each participant using standardized protocols as described. All body measurements are recorded on our Body Measurement Form, and are periodically key-punched into an ACCESS data base.

Stage 3: Data Entry, Verification and Interim Analysis, Months 12-30

1. Assure that all data are immediately read into analytic databases

Year 2: Data from the diet and physical activity interviews are immediately available for analyses. Data reported by questionnaire, or recorded on the appropriate forms, have been periodically key-punched into ACCESS databases, as work loads permit.

Year 3: Data entry for analytic study database continues.

Year 4: Data entry for analytic study database is complete for current recruits.

2. Flag all outlier and illogical responses

Year 2: All questionnaires are reviewed prior to data entry to identify possible illogical responses. At the time of this report, study recruitment and data collection are in progress. Once recruitment and data collection are closed, data verification and quality control procedures will be implemented.

Year 3: Outliers and illogical responses were flagged. Questionnaires were checked to confirm or correct values.

Year 4: Outliers and illogical responses were flagged. Questionnaires were checked to confirm or correct values.

3. Verify all outlier and illogical responses, re-contacting participants, if necessary.

Year 3: Patients were contacted as necessary to resolve any discrepancy.

4. Conduct simple descriptive analyses (e.g., cross-tabulations and univariate statistics)

Year 3: Simple descriptive analyses completed.

Year 4: Multivariable analyses are completed.

Stage 4: Laboratory Analyses, Months 24 – 30

1. Transfer urine and blood samples to the lab of Dr. Parl for estradiol and estrogen metabolite assays

Year 2: A subset of urine specimens have been transferred to Dr. Parl's lab for preliminary testing of endocrine assays. This will help us evaluate assay quality and variability when urine specimens, such that this analysis may be more rapidly completed for all subjects in Year 3 of the project.

Year 3: Urine samples are available to Dr. Parl's lab. Samples have not been analyzed at this time due to personnel changes in the lab. However, it remains our goal to analyze these samples in accordance with the initial application. Also, we note that the research in the area of urinary estrogen metabolite measurement has progressed, and a post-doc in Dr. Parl's lab is update all analytic procedures to meet these improvements.

Year 4: We have pursued alternative opportunities to measure estrogen metabolite levels with Genova Diagnostics (Ashville, NC). Data should be available soon. Additionally, we have measured blood leptin levels because our preliminary analyses showed a strong relationship between PIN and obesity, and this was determined to be a priority area of research by the NCI and others.

2. Transfer blood sample to lab of Dr. Parl for estradiol assay

Year 3: see above

Year 4: We are at present seeking funding from the NCI to conduct estradiol analyses on a much larger study population sample.

3. Transfer blood samples to lab of Dr. Cai for CYP1B1 and CYP3A4 genotyping assays

Year 3: DNA was extracted from 336 blood samples, including 60 HGPIN patients, prostate cancer patients, and controls. A single 396 well plate was prepared by the Vanderbilt Genetics Core Laboratory, using 0.5 ug DNA were well from 378 subjects. Analysis included 147 cancer patients, 52 HGPIN patients, and 179 controls, frequency matched by age (5 year categories) and race. Genotyping for CYP1B1 L/V was performed by TaqMan assay. Genotyping for CYP3A4 will use this same plate. CYP3A4 genotyping is in progress, and analytic results are expected in 2-4 weeks.

Year 4: Genotyping protocols have been completed (see below). Indeed, the genotyping protocol was so successful that we added PPAR γ 2, PPAR α , and CYP19 genotyping protocols to continue to investigate the genetic effects of estrogens on HGPIN status. Body fat mass (adiposity) is the primary source of estrogens in men, and we have also added

measurements of blood leptin as a biomarker of body fat mass consistent with our primary aims. PSA also has been assessed.

4. Confirm quality control procedures, and repeat assays if necessary

Stage 5: Final Data Analysis, Months 30-36:

1. Perform exploratory analyses to test for adherence to model assumptions

Year 3: Exploratory analyses in progress, but at this time all modeling assumptions and other assumptions necessary for statistical analyses appear to be met.

Year 4: Exploratory analyses have been conducted according to protocol.

2. Perform any necessary data transformations to meet statistical assumptions

Year 3: Variables reflecting weight, BMI, WHR, and other anthropometric parameters are scales as continuous variables and also categorized to evaluate inconsistent relationships.

Year 4: Data were transformed as necessary.

3. Test study hypothesis

Year 3: The primary analysis were in progress.

Year 4: Primary statistical analyses have been performed according to protocol. Results are summarized below.

4. Conduct post-hoc analyses of study data

Year 3: We have conducted several post hoc analyses looking at the relationship between obesity and prostate cancer. Also, we have extended our genetic analyses to include genetic polymorphisms associated with obesity and prostate carcinogenesis (PPAR- γ , PPAR- α , CYP19).

Year 4. We continued our post hoc analyses of obesity and prostate cancer risk. Results are summarized below.

Subjects included patients receiving a diagnostic biopsy and ultimately diagnosed with cancer, HGPIN, or without cancer or HGPIN (i.e. biopsy negative or B-Neg controls). We analyze data to evaluate our hypotheses regarding the role of genetic markers of estrogen exposure and prostate cancer progression and HGPIN risk. All subjects consented to provide three tubes of blood and a urine collection. Whole blood, serum, plasma, and urine were aliquoted into cryovials and frozen at -80° C. We record time of day for blood collection and time since last meal. Clinics schedule biopsy procedures in the morning, and 62% of blood samples were collected between 8:00

a.m. and 12:00 p.m. Only 3% of samples were collected after 3:00 pm. Over 50% of men avoided food for at least 6 hours before blood collection and the biopsy. Thus, blood biomarker analyses will be able to control for variability due to circadian rhythm or fasting status.

Age ranged from 40 to 89 years, and averaged 65.3 years. About 6% of subjects were less than age 50, while 52% were 65 years old or older. B-Neg controls were about 2 years younger ($p < 0.05$) than cancer or PIN cases (64.0, 66.4, 66.3 years, respectively).

Table 1 summarized descriptive data potentially leading to prostate cancer screening and

Table 1: Screening Factors		Cancer	PIN	B-Neg	p*
Race	African-Amer.	11%	11%	10%	0.66
Marital	married/part.	84%	88%	86%	0.68
Fam. Hx.	prostate can.	21%	35%	24%	0.06
Tobacco	Current	18%	20%	19%	0.94
Tobacco	Ever	67%	67%	68%	0.98
Education	≤ H.S.	44%	39%	43%	0.93
	Any Coll	40%	43%	39%	
	Grad. Sch.	16%	18%	18%	
Employed	not empl.	29%	34%	36%	0.52
	full-time	9%	10%	6%	
	part-time	62%	57%	58%	
Insurance	Medicare	45%	47%	35%	0.33
	Medicaid	4%	3%	3%	
	HMO	9%	8%	13%	
	PPO/BC	23%	25%	29%	
	Other	16%	17%	17%	
	None	4%	0%	2%	

* All P values age adjusted.

prostate cancer detection. Overall, approximately 11% were African-American, and almost 85% were married or living with a partner. About 25% has a family history of prostate cancer, about two-thirds has ever used tobacco, and about 50% had attended college at some level. Most subjects maintained a partial employment level and were covered by some form of health insurance. These screening determinants, including race, income, education, marital status, and employment, did not significantly differ across groups. Family history was marginally greater among PIN cases, but did not differ between cancer cases and B-Neg controls.

Table 2 summarizes PSA levels and

prostate cancer screening practices. Logically, PSA among cancer cases was higher, although the

Table 2: Prostate Cancer Screening (Medical Charts)				
	B-Neg.	PIN	Cancer	p
PSA (ng/ml)	4.9±1.0	6.0±1.0	6.9±1.0	<0.01
Days until Biopsy	51±4.9	47±8.9	53±5.6	0.69
# PSA tests	2.1±0.1	2.2±0.1	1.9±0.1	<0.01
DRE positive	5%	2%	10%	0.01
BPH symptoms	85%	85%	87%	0.59

difference not large. PSA levels did not significantly differ between B-Neg and PIN subjects ($p = 0.10$). Just 44 subjects had a positive DRE, including two PIN cases and 18 B-Neg controls. The time between the last PSA test and biopsy were comparable across groups, suggesting

delayed diagnosis does not affect study results. Furthermore, the prevalence of lower urinary tract symptoms leading to BPH were similar between groups. Cancer cases received fewer PSA tests prior to diagnosis, as PSA testing ends with diagnosis.

The distribution of candidate genetic polymorphisms in CYP1B1 and CYP3A4 are summarized in Table 3. We also considered relevant SNPs in PPARs. A CYP19 polymorphism previously observed to be associated with breast cancer was not prevalent and not considered for further analysis.

Table 3: Distribution of candidate genetic polymorphisms.					
Gene	CYP1B1	CYP3A4	PPAR- α	PPAR γ 2	CYP19
SNP	Leu432Val	A – G	Leu162Val	Pro12Ala	Tyr39Arg
Ref	11,12	6	13-15	16-18	19-21
Alleles	LL: n=110 (29%) LV: n=167 (44%) VV: n=99 (26%)	AA: n=312 (85%) AG: n=43 (12%) GG: n=12 (3%)	LL: n=323 (86%) LV: n=50 (14%) VV: n=1 (0%)	PP: n=304 (82%) PA: n=65 (17%) AA: n=4 (1%)	TT: n=370 (100%) TA: n=1 AA: n=3

Table 4 summarizes the association between genetic polymorphisms and risk of HGPIN, Cancer, or Advanced Cancer (Gleason ≥ 7). Logistic regression was used to calculate Odds Ratios (OR) and 95% confidence intervals (95% CI), adjusted for age, PSA, BMI, and WHR. No genetic variant was significantly associated with HGPIN, cancer, or advanced cancer.

Table 4		B-Neg	HGPIN		Cancer		Adv. Cancer	
CYP1B1	LL	46 (26%)	18 (35%)	1.0 (ref)	46 (32%)	1.0 (ref)	16 (29%)	1.0 (ref)
	LV	86 (49%)	20 (38%)	0.64 (0.30, 1.38)	59 (41%)	0.70 (0.54, 1.88)	27 (49%)	0.90 (0.40, 2.00)
	VV	44 (25%)	14 (27%)	1.13 (0.48, 2.69)	39 (27%)	1.02 (0.54, 1.88)	12 (22%)	0.89 (0.34, 2.29)
	LV+VV	130 (74%)	34 (65%)	0.78 (0.39, 1.57)	98 (68%)	0.81 (0.48, 1.34)	39 (71%)	0.89 (0.42, 1.90)
CYP3A4	AA	154 (86%)	45 (87%)	1.0 (ref)	124 (86%)	1.0 (ref)	47 (85%)	1.0 (ref)
	AG+GG	25 (14%)	7 (13%)	1.21 (0.48, 3.07)	21 (14%)	0.91 (0.46, 1.82)	8 (15%)	0.63 (0.20, 1.95)
PPAR- α	LL	154 (88%)	47 (90%)	1.0 (ref)	119 (84%)	1.0 (ref)	47 (87%)	1.0 (ref)
	LV +VV	22 (12%)	5 (10%)	0.94 (0.33, 2.71)	23 (16%)	1.49 (0.77, 2.90)	7 (13%)	1.18 (0.42, 3.30)
PPAR γ 2	PP	140 (80%)	40 (77%)	1.0 (ref)	121 (85%)	1.0 (ref)	44 (83%)	1.0 (ref)
	PA+AA	35 (20%)	12 (23%)	1.00 (0.46, 2.21)	21 (15%)	0.70 (0.38, 1.29)	9 (17%)	0.80 (0.33, 1.94)

adjusted for age, PSA, body mass index, and waist-to-hip ratio

Estrogens in men are produced by androgen aromatization by cytochrome P-450 19 (CYP19) expressed predominately in adipocytes (176). With age, body adiposity increases, and thus estrogen levels increase with age and obesity while T levels decrease. This is a fundamental shift in steroid composition with age and obesity (31;177-179). Thus, we next extended the investigation to measures of body adiposity and prostate cancer.

Table 5 summarizes the body size and prostate cancer or HGPIN associations. No body size measure was significantly associated with total cancer risk. However, several measures were related to HGPIN. BMI (continuous) was associated with reduced HGPIN risk (OR = 0.93 (0.87, 0.99), as did the trend for increasing BMI categories ($p_{\text{trend}} > 0.05$). Blood leptin levels also were significantly associated with reduced HGPIN risk (OR_{adj}=0.96 (0.92, 0.99)), and provides a measure of body adiposity independent of the known errors associated with BMI in older men. The WHR was significantly associated with HGPIN, such that men in the highest quartile of the WHR experience a 3-fold increased risk of HGPIN ($p_{\text{trend}} = 0.004$). Removing BMI from the model did not substantively alter interpretation. Associations between HGPIN and height, sitting height, or leg length were marginal or without trend, but will be explored in future analyses with a larger sample size.

To minimize possible sampling error, we stratified HGPIN analyses by PSA and age (**Table 6**). Over-stratification led to unstable estimates, but the directions of effects were consistent across age and PSA. A very recent analysis of prostate biopsy patients suggested prostate volume may confound associations²². We have since started reviewing surgical records (in addition to

pathology records) to capture volumes assessed by ultrasound, and volume data are recorded for about 90% of subjects. Data are available from 200 subjects. PSA was significantly correlated with volume ($r_s=0.24$, $p<0.01$), but not BMI ($r_s = 0.08$), WHR ($r_s=-0.01$) or leptin ($r_s = 0.00$). Although sample size and thus precision are greatly reduced, the associations between HGPIN and BMI ($OR_{adj} = 0.95$ (0.88, 1.03)) or WHR ($OR_{Q4vs Q1, adj} = 2.44$ (0.94, 6.31)) remain largely unchanged. The effect of prostate volume on analyses will be carefully investigated in the full study.

Table 7 summarizes data on obesity and advanced disease diagnosis. BMI and leptin levels were significantly associated with aggressive cancer risk among men over 70 years of age. Estimated visceral adipose tissue (WHR) was not associated with advanced stage disease. No obesity index was associated with Gleason 6 cancer.

		B-Neg. N (%)	N (%)	HGPIN OR* (95%CI)	N (%)	Cancer OR* (95%CI)
BMI**	< 25	66 (19)	20 (20)	1.0	59 (23)	1.0
	25 - <30	175 (51)	58 (57)	0.86 (0.45, 1.62)	117 (45)	0.75 (0.47, 1.21)
	30- < 35	76 (22)	19 (19)	0.55 (0.24, 1.28)	65 (25)	1.01 (0.57, 1.81)
	≥ 35	26 (8)	5 (5)	0.43 (0.13, 1.38)	21 (8)	0.89 (0.41, 1.91)
BMI	< 25.5	87 (25)	27 (26)	1.0	76 (28)	1.0
	25.5 - < 27.9	86 (25)	33 (32)	1.15 (0.62, 2.17)	61 (23)	0.97 (0.58, 1.59)
	27.9 - < 30.5	84 (24)	21 (20)	0.58 (0.28, 1.20)	60 (22)	0.97 (0.58, 1.62)
	> 30.5	88 (26)	22 (21)	0.62 (0.29, 1.32)	72 (27)	1.19 (0.70, 2.03)
WHR	<0.95	86 (25)	19 (18)	1.0	70 (27)	1.0
	0.95 - <1.00	72 (21)	21 (20)	1.59 (0.75, 3.36)	47 (18)	0.75 (0.45, 1.28)
	1.00- <1.04	100 (29)	26 (25)	1.52 (0.72, 3.20)	68 (26)	0.77 (0.47, 1.26)
	≥1.04	86 (25)	37 (36)	3.19 (1.50, 6.76)	74 (29)	0.96 (0.56, 1.63)
Waist (cm)	< 96.5	98 (28)	30 (29)	1.0	82 (32)	1.0
	96.5 - < 102.9	72 (21)	21 (20)	1.13 (0.55, 2.28)	51 (20)	0.79 (0.48, 1.32)
	102.9 - <111.8	99 (29)	32 (31)	1.51 (0.71, 3.19)	69 (27)	0.83 (0.49, 1.41)
	≥ 111.8	75 (22)	20 (19)	2.02 (0.71, 5.72)	57 (22)	0.81 (0.40, 1.65)
Hips (cm)	< 99.0	78 (23)	28 (27)	1.0	75 (29)	1.0
	99.0 - < 104.1	90 (26)	28 (27)	0.94 (0.49, 1.83)	60 (23)	0.65 (0.39, 1.08)
	104.1- <109.2	91 (26)	24 (23)	0.78 (0.37, 1.67)	54 (21)	0.59 (0.34, 1.00)
	≥ 109.2	85 (25)	23 (22)	1.02 (0.39, 2.65)	70 (27)	0.78 (0.40, 1.48)
Height (cm)	< 168.9	87 (25)	22 (22)	1.0	60 (23)	1.0
	168.9 - < 174.0	93 (27)	18 (18)	0.82 (0.39, 1.72)	82 (31)	1.36 (0.85, 2.19)
	174.0 - < 179.1	81 (24)	40 (39)	2.65 (1.37, 5.12)	55 (21)	1.30 (0.78, 2.16)
	≥ 179.1	82 (24)	22 (22)	1.34 (0.65, 2.76)	65 (25)	1.38 (0.83, 2.29)
Sit Hgt (cm)	< 86.7	85 (25)	20 (20)	1.0	69 (27)	1.0
	86.7 - < 89.5	81 (24)	28 (27)	1.72 (0.87, 3.39)	67 (26)	1.25 (0.77, 2.02)
	89.5 - < 92.7	86 (25)	25 (25)	1.35 (0.66, 2.75)	53 (20)	0.94 (0.57, 1.57)
	≥ 92.7	90 (26)	29 (28)	1.84 (0.91, 3.74)	70 (27)	1.28 (0.78, 2.09)
Legs (cm)	< 81.3	93 (27)	23 (22)	1.0	71 (26)	1.0
	81.3 - <85.1	87 (25)	28 (27)	1.65 (0.84, 3.26)	68 (25)	1.39 (0.85, 2.25)
	85.1 - <89.4	80 (23)	30 (29)	1.81 (0.92, 3.58)	66 (25)	1.39 (0.85, 2.28)
	> 89.4	85 (25)	22 (21)	1.12 (0.54, 2.30)	64 (24)	1.12 (0.67, 1.85)

* Adj. for age (continuous), BPH (Y/N), PSA level (continuous), # prior PSA tests (continuous), BMI (continuous), and race (white/non-white). ** Adj. for age (continuous), BPH (Y/N), PSA level (continuous), # prior PSA tests (continuous), WHR (continuous), family hx, and race.

Table 6: Obesity and HGPIN, by age or PSA					
		Age		PSA	
		< 70 yrs	≥ 70 yrs	< 7	≥ 7
BMI	< 25.5	1.0	1.0	1.0	1.0
	25.5 - < 27.9	1.37 (0.62, 3.03)	0.55 (0.17, 1.78)	1.08 (0.49, 2.36)	1.50 (0.49, 4.51)
	27.9 - < 30.5	0.85 (0.35, 2.07)	0.13 (0.03, 0.59)	0.59 (0.25, 1.41)	0.51 (0.12, 2.15)
	> 30.5	0.74 (0.29, 1.85)	0.48 (0.12, 1.94)	0.67 (0.27, 1.69)	0.54 (0.14, 2.13)
WHR	<0.95	1.0	1.0	1.0	1.0
	0.95 - <1.00	1.43 (0.59, 3.48)	2.30 (0.52, 10.0)	0.99 (0.40, 2.42)	5.72 (1.16, 28.3)
	1.00 - <1.04	1.61 (0.67, 3.82)	1.51 (0.34, 6.62)	1.62 (0.69, 3.82)	1.89 (0.39, 9.13)
	≥1.04	1.82 (0.73, 4.54)	11.3 (2.55, 50.2)	2.42 (1.03, 5.67)	7.81 (1.49, 40.9)

Table 7: Obesity and Aggressive Cancer (Gleason ≥ 7, n=114)			
		Age	
		All Subjects	≥ 70 yrs
BMI	< 25.5	1.0	1.0
	25.5 - < 27.9	1.14 (0.57, 2.27)	2.39 (0.84, 6.76)
	27.9 - < 30.5	0.96 (0.46, 1.98)	1.33 (0.44, 4.04)
	> 30.5	1.46 (0.71, 3.08)	3.50 (1.01, 11.67)
Leptin	< 5.5	1.0	1.0
	5.5 - <8.2	0.98 (0.46, 2.04)	1.77 (0.53, 5.81)
	8.2 - <12.7	1.94 (0.96, 3.91)	2.91 (0.93, 9.12)
	≥12.7	1.24 (0.59, 2.59)	2.56 (0.81, 8.01)
WHR	<0.95	1.0	1.0
	0.95 - <1.00	1.34 (0.5, 3.08)	1.36 (0.32, 5.84)
	1.00 - <1.04	1.30 (0.62, 2.74)	1.27 (0.41, 3.99)
	≥1.04	1.04 (0.48, 2.27)	0.87 (0.25, 3.00)

In summary, we present a unique opportunity to recruit subjects and collect high-quality data to address questions of public health significance, including the relationship between obesity and prostate cancer. In Nashville, men are referred to one of four clinics for prostate cancer diagnosis, and recruitment from these clinics is efficient and productive. Recruitment and data collection protocols have been streamlined to permit rapid recruitment. Adipocytes (i.e., fat cells) regulate insulin sensitivity, steroid hormone metabolism, inflammatory responses, and release leptin and other adipokines. Prostate cells respond to each of these processes, yet the obesity and prostate cancer relationship remains unclear. Results from our pilot study provide the first evidence that visceral adiposity, estimated by the waist-to-hip ratio (WHR), is significantly associated with the prostate cancer precursor high-grade prostatic intraepithelial neoplasia (HGPIN) ($OR_{adj}=3.19$, 95% CI (1.50, 6.76), $p_{trend}=0.004$). Visceral adiposity controls insulin and inflammatory pathways, suggesting metabolic instability or low-grade inflammation impacts early stages of prostate carcinogenesis. Also, estimated total body adiposity, by the body mass index (BMI), was significantly associated with reduced HGPIN risk (BMI continuous: $OR_{adj}=0.93$ (0.92, 0.99)). Furthermore, BMI was associated with aggressive cancer, particularly after age 70 years (BMI_{Q4} vs Q1: $OR_{adj}=3.50$ (1.01, 11.67)). This suggests an estrogen-rich environment has two effects -

inhibition during earlier phases but advancement to clinically relevant disease. Our ongoing investigation will consider the pathways through which obesity advances and inhibits prostate carcinogenesis. Preliminary analyses found BMI and the WHR associated with HGPIN, but in opposing directions, while BMI also was associated with advanced disease.

5. Prepare manuscripts

Year 3: Manuscript preparation has not yet started, but we anticipate several manuscripts from this work.

Year 4: Three manuscripts has been submitted for publication.

6. Archive datasets for future analyses and future patient follow-up

Year 3: All datasets are backed-up daily on the server for security and storage

Year 4: All datasets are backed-up daily on the server for security and storage

7. Plan for future studies

Year 3: This work has motivated several ongoing, submitted, and proposed grant proposals.

Year 4: An NCI grant proposal has been submitted in response to preliminary data developed through this work. .

KEY RESEARCH ACCOMPLISHMENTS in Year 4

- IRB approvals maintained
- Manual of Operations maintained
- Participant recruitment, including consent, recruitment, eligibility, data collection, biospecimen collection, and storage protocols.
- Exceed all recruitment and data collection projections.
- All pathology data abstraction complete
- Initiate surgical record review to determine prostate volume
- Data-entry of all questionnaire data, and evaluation of data quality
- Completed genotyping of CYP1B1 and CYP3A4
- Extended genotyping to PPAR- γ 2, PPAR- α , and CYP19 genes, exceeding the aims of the original protocol
- Obtained funding from NCI to investigate obesity and prostate cancer
- Obtained funding from American Institute of Cancer Prevention to investigate effects of diet change on prognosis following surgery.
- Obtained funding from Prostate Cancer Foundation to investigate role of diabetes and insulin sensitivity on prostate cancer risk.
- Less tangible, but no less significant, this study initiated a prostate cancer research program across Vanderbilt and Meharry Medical College (an HBCU) that has led to the funding of a Prostate Cancer Research Center from the DOD.
- Submission for NCI funding to investigate obesity and HGPIN, potentially leading to important

public health interventions to delay the progression of prostate cancer to clinically relevant disease.

- Several publications have been submitted or are in progress.

REPORTABLE OUTCOMES

Subsequent Grant Submissions

Many grant applications have been based on this project, including .

1. A pilot project in a Prostate Cancer SPORE application submitted to the NIH. The objective was a case-control study extending this recruitment protocol to investigate additional biomarkers of prostate cancer risk (SPORE was not funded).
2. A pilot intervention to the American Institute of Cancer Research to investigate the effects of diet on PSA levels among prostate cancer patients. Protocols and recruitment developed via the NIA were critical in this application. This project was funded, and we are currently running the protocol
3. Dr. Fowke submitted an IDEA award to the DOD to investigate the effects of indole-3-carbinol or Crucifer vegetable intake among men with PSA recurrence following prostatectomy (unfunded).
4. Dr. Fowke submitted an exploratory project to the DOD investigating serologic markers of obesity in relation to PSA recurrence following prostatectomy. This received a priority score of 1.8 and was unfunded.
5. Dr. Fowke has obtained supplemental pilot funding from the NCI to extend the investigation of obesity and prostate cancer (R21 CA98348).
6. Dr. Fowke was recently listed among collaborators in a DOD funded Prostate Cancer Research Center grant, awarded to Meharry Medical School (PI: F. Ukoli). Dr. Ukoli and Dr. Fowke are collaborating on Dr. Ukoli's initiatives to conduct a diet intervention among African-American men, and Dr. Ukoli will conduct analyses using data collected under this protocol in the future to provide preliminary data for future grant applications.
7. In June, 2005, Dr. Fowke submitted an RO1 to the NCI proposing to investigate the genetic and endocrine markers of body adiposity in relation to HGPIN and prostate cancer. This proposal has been resubmitted following response to reviewer comments.

Posters

Body adiposity and HGPIN and Prostate Cancer. Frontiers in Cancer Prevention, 2005.

Papers Submitted for publication

1. Urine proteome MALDI profiling for detection of high-grade prostatic intraepithelial neoplasia (HGPIN) and prostate cancer, A. M'Koma, J. Fowke, et al. Clinical Chemistry.
2. Indicators of body adiposity and PSA, Fowke et al. Urology
3. Body adiposity and high grade prostatic intraepithelial neoplasia, Fowke et al, Urology

CONCLUSIONS

Recruitment and data collection protocols were fully completed. Indeed, we have exceeded every recruitment goal, enabling new analyses of HGPIN. We have data and biospecimen collection from 717 subjects, and 95% of eligible subjects approached for recruitment consented to participate in this study. This is a very respectable recruitment rate. We note that one laboratory assay has not been completed at this time because of difficulty in finding trained personnel to carry out the assay. However, we also note that we are making arrangements with a commercial vendor (Genova Diagnostics, Ashville, NC) to meet this objective. Also, this NIA provided the opportunity for Dr. Fowke to submit several prostate cancer research grants to the DOD, NIH, and other agents. Two awards utilized data from this project. Three publications have been submitted at this time, and we anticipate continued publication from these datasets. Statistical analyses will continue to investigate hypotheses linking steroid hormone metabolism to prostate cancer progression.

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